

Remarks

Upon entry of the above amendment, Claims 55 to 84, 106 to 111, 113, 114, and 130 to 148 are pending in the application, with 55, 56, 71, 72, 106, 113 and 136 being the independent claims. Claims 1 to 54, 85 to 105, 112, and 115 to 129 are sought to be cancelled without prejudice or disclaimer of the subject matter therein. Amendment to claims 55 to 57, 61 to 74, 78, 80 to 84, 106, 109, 113, 130, and 135 is sought. New claims 136 to 148 are sought to be added.

Amended claims 55 to 57, 61 to 74, 78, 80 to 84, 106, 109, 113, 130, and 135 correspond to claims 55 to 57, 61 to 74, 78, 80 to 84, 106, 109, 113, 130, and 135 of the parent application, 09/984,664, filed October 30, 2001, and correspond to Restriction Group V identified in the Office Action of March 13, 2003. Applicant has amended the claims to correct minor typographical or grammatical errors, and to make the language of the claims consistent throughout the present application. The subject matter of the amended claims is the same as the original claims, and introduce no new matter. Consideration and entry of the amended claims is respectfully requested.

New claims 136 to 148 are supported by the specification at *inter alia*, pages 1, 4 to 6, 9 to 14, 16, 24 to 26, 32, 37, 42 to 44, 48, 57, 70, 71, 76, 78, 79 and 84 to 93.

These new claims are believed to introduce no new matter, and their entry is respectfully requested.

Amendment to the Drawings

The deletion of Figures 29A, 29B and 29C is sought.

Changes are sought to correct obvious defects in the informal drawings, prior to submission of the formal drawings. Attached herewith is a "Request to Approve Proposed Drawing Corrections," copies of 4 sheet(s) of drawings, containing proposed corrections to Figures 3, 13, 14, and 15 shown in red. For comparison purposes, unmarked original and amended drawings are also provided. The proposed changes add no new matter to this application.

In Figure 3, the letter H had been overlaid on O, C, and N, obscuring the identity of the functional group. It is obvious to one of ordinary skill in the art that the correct functional groups are OH, CH₂, and NH or NH₂ respectively. Similarly, the negative charge sign (-) was incorrectly overlain upon certain O groups. The (-) has been moved so that the "O-" can be clearly seen.

In Figure 13, the 11th letter in the second methylated strand should obviously be a letter G, not an A, corresponding to the letter G at the same position in the other 3 strands shown. Essentially the same nucleotide sequence is found in Figures 13 through 15.

In Figure 14, obvious errors in the sequence have been corrected to ensure that the sequences are the same in Figures 13, 14 and 15. To that end, the 11th letter in both strands is changed from A to G; the 22nd letter in the deaminated unmethylated DNA has been changed from G to A; and the fifth letter in the deaminated methylated DNA has been changed from Me-U to Me-C.

In Figure 15 it is clear that the nucleotide in the left and right strands do not correctly align. The DNA sequence in the left strand has now been shifted down one nucleotide, so that all nucleotides correctly align.

All of the above errors were most likely introduced as a result of an error by the original draftsman of the informal drawings and have been corrected in the replacement sheets of drawings. These corrections are sought to bring the drawings into conformity with the description.

Figures 29A, 29B and 29C have been cancelled. This cancellation has no effect on the patentability of the claims and thus are not required for written description or enablement support. To correctly number the subsequent Figures, renumbering of Figures 30 and 31 is sought. These changes introduce no new matter.

Applicant requests that the Examiner approve the proposed corrections. After official communication of such approval, Applicants will submit appropriately corrected formal drawings

Amendment to the Specification

In order for the specification to conform with the drawings following the deletion of Figures 29A, 29B and 29C, Applicant seeks amendment of the specification at paragraphs [0069] through [0071], and at [0244]. These amendments remove reference to the cancelled Figures, and renumber the subsequent Figures.

Amendment to the specification at the “CROSS REFERENCE TO RELATED APPLICATIONS” is sought to claim priority to a prior application.

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To Be Assigned (Div. of Appl.
No. 09/984,664; Filed: October 30, 2001)

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicant believes the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

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Version with markings to show changes made

In the Drawings

Figures 29A, 29B and 29C are deleted. Figures 30 and 31 are therefore renumbered 29 and 30, respectively. Informal drawings are substituted with the formal drawings. Correction of obvious defects is sought in Figures 3, 13, 14 and 15, substituting informal Figures 3, 13, 14 and 15 with Figures 3, 13, 14 and 15 in the attached formal drawings.

In the Specification

The CROSS REFERENCE TO RELATED APPLICATIONS is new.

Paragraph [0069] is deleted.

Paragraphs [0070] and [0071] are amended as follows:

[0070] [FIG. 30.] FIG. 29. Portion of the contig sequence of the CDKN2A gene. The sequence represents a small portion of the contig starting at 856630 nucleotides from the start of the contig sequence. The sequence represents a CpG island. Contig number: NT_008410.4.

[0071] [FIG. 31.] FIG. 30. Schematic representation of a “capture probe” to determine the methylation status of a specific gene. Oligonucleotide probes that are specific for a region near the CpG island of the target gene are immobilized onto a microtiter plate. The DNA of interest is added to the immobilized probe and bound to the capture probe. The DNA is then chemically modified to convert unmethylated C to T, and leave methyl-C unaffected. The converted DNA can then be amplified by an optional PCR step to further enhance the signal. A labeled CpG initiator is then added with an RNA polymerase and labeled nucleotide(s).

Paragraph [0244] is amended as follows:

[0244] [As shown in Figure 29A, as] As the oligonucleotide product is generated, energy transfer occurs between TAMARA-SpApG and SF-UTP, which changes the wavelength at which TAMARA emits. If RNA polymerase or DNA is omitted from the reaction, there is no transfer of energy between the initiator and the terminator, and no change in the wavelength at which TAMARA emits [(Figure 29B and 29C)].

In the Claims

Claims 1 to 54, 85 to 105, 112, and 115 to 129 are cancelled. Claims 136 to 148 are new.

The following 55 to 57, 61 to 74, 78, 80 to 84, 106, 109, 113, 130, and 135 are amended as follows:

55. A method for detecting DNA or RNA in a test sample, said method comprising:
 - (a) hybridizing a single stranded target polynucleotide with an abortive promoter cassette comprising a sequence that hybridizes to the single stranded target polynucleotide, and a region that can be detected by transcription by a polymerase;
 - (b) incubating said target polynucleotide with an RNA[-]polymerase, an initiator, and a terminator;
 - (c) synthesizing an oligonucleotide transcript that is complementary to the initiation start site of [the APC] said abortive promoter cassette, wherein said initiator is extended until said terminator is incorporated into said [oligonucleotides]oligonucleotide transcript, thereby synthesizing multiple reiterative oligonucleotide transcripts; and
 - (f) detecting or quantifying said [reiteratively synthesized] reiterative oligonucleotide transcripts.

56. A method for detecting the presence of pathogens in a test sample, said method comprising [the steps of]:
 - (a) hybridizing a single stranded target pathogen polynucleotide in said test sample with an abortive promoter cassette comprising a region that can be detected by transcription by a polymerase;
 - (b) incubating said target polynucleotide and an initiator with an RNA[-]polymerase, and a terminator;
 - (c) synthesizing an oligonucleotide transcript that is complementary to initiation start site of the [APC] abortive promoter cassette, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple abortive reiterative oligonucleotide transcripts; and
 - (d) determining the presence of a pathogen by detecting or quantifying said [reiteratively synthesized] reiterative oligonucleotide transcripts synthesized from said test sample.
57. The method of any one of claims [54-56] 55 or 56, further comprising detecting or quantifying said reiteratively synthesized oligonucleotide transcript by modifying a nucleotide in at least one of the members selected from the group consisting of said terminator, and said initiator.
61. The method of any one of claims [54-56] 55 or 56, wherein said polymerase is selected from the group consisting of: a DNA-dependent RNA polymerase, an RNA-dependent RNA polymerase and a modified RNA polymerase, and a primase.
62. The method of claim 61, wherein said polymerase comprises an RNA polymerase derived from one of [E. coli, E. coli] E. coli, E. coli bacteriophage T7, [E. coli] E. coli bacteriophage T3, and [S. typhimurium] S. typhimurium bacteriophage SP6.

63. The method of any one of claims [54-56] 55 or 56, wherein said abortive oligonucleotides being synthesized are one of the lengths selected from the group consisting of: about 2 to about 26 nucleotides, about 26 to about 50 nucleotides and about 50 nucleotides to about 100 nucleotides.
64. The method of any one of claims [54-56] 55 or 56, wherein said chain terminator comprises a nucleotide analog.
65. The method of any one of claims [54] 55 or 56, wherein said initiator comprises nucleotides selected from the group consisting of: 1-25 nucleotides, 26-50 nucleotides, 51-75 nucleotides, 76-100 nucleotides, 101-125 nucleotides, and 126-150 nucleotides, 151-175 nucleotides, 176-200 nucleotides, 201-225 nucleotides, 226-250 nucleotides, and greater than 250 nucleotides.
66. The method of any one of claim 55 or 56, wherein said single-stranded target polynucleotide is one of DNA and RNA.
67. The method of any one of claims [54-56] 55 or 56, wherein said initiator is [one of] RNA.
68. The method of claim 55 or 56, wherein said initiator comprises nucleotides selected from the group consisting of: 1-25 nucleotides, 25-50 nucleotides, 50-75 nucleotides, 75-100 nucleotides, 100-125 nucleotides, and 125-150 nucleotides, 150-175 nucleotides, 175-200 nucleotides, 200-225 nucleotides, and 225-250 nucleotides.
70. The method of any one of claim 55 or 56, wherein said abortive promoter cassette further comprises an [APC] abortive promoter cassette linker which is adapted to hybridize to an end of said target pathogen polynucleotide.
71. A method for detecting pathogens in a test sample, said method comprising:

- (a) immobilizing a capture probe designed to hybridize with a target [DNA] polynucleotide in said test sample;
- (b) hybridizing said capture probe with a test sample that potentially contains said target [DNA] polynucleotide;
- (c) hybridizing a single stranded target [DNA] polynucleotide in said test sample with an abortive promoter cassette comprising a region that hybridizes to the single stranded target pathogen polynucleotide, and a region that can be detected by transcription by a polymerase;
- (d) incubating said target polynucleotide with an RNA-polymerase, initiator, and a terminator;
- (e) synthesizing an oligonucleotide transcript that is complementary to the [said initiation transcription] transcription initiation start site of said [APC] abortive promoter cassette, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple reiterative oligonucleotide transcripts; and
- (f) determining the presence or absence of a pathogen by detecting or quantifying said [reiteratively synthesized] reiterative oligonucleotide transcripts.

72. A method for detecting mRNA expression in a test sample, the method comprising:

- (a) hybridizing a target mRNA sequence with an abortive promoter cassette comprising a region that can be detected by transcription by a polymerase;
- (b) incubating said target mRNA sequence with an RNA-polymerase, an initiator, and a terminator;
- (c) synthesizing an oligonucleotide transcript that is complementary to the transcription initiation start site, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript, thereby synthesizing multiple reiterative oligonucleotides; and
- (d) determining the presence or absence of the mRNA by detecting or quantifying said [reiteratively synthesized] reiterative oligonucleotide transcripts [synthesized from said test sample].

74. The method of claim 72, wherein modifying further comprises incorporating an independently selected label moiety into at least one of said initiator, said terminator, and said oligonucleotides.
78. The method of claim 72, wherein said polymerase comprises an RNA polymerase derived from one of [E. coli, E. coli] E.coli, E. coli bacteriophage T7, [E. coli] E. coli bacteriophage T3, and [S. typhimurium] S. typhimurium bacteriophage SP6.
80. The method of claim 79, wherein said initiator comprises nucleotides selected from the group consisting of: 1-25 nucleotides, 26-50 nucleotides, 51-75 nucleotides, 76-100 nucleotides, 101-125 nucleotides, [and] 126-150 nucleotides, 151-175 nucleotides, 176-200 nucleotides, 201-225 nucleotides, 226-250 nucleotides, and greater than 250 nucleotides.
81. The method of claim 72, wherein said abortive oligonucleotides being synthesized are one of the lengths selected from the group consisting of: about 2 to about 26 nucleotides, about 26 to about 50 nucleotides [and] about 50 nucleotides to about 100 nucleotides, and greater than 100 nucleotides.
83. The method of claim 72, wherein said abortive promoter cassette comprises an abortive promoter cassette [APC] linker which is adapted to hybridize to a poly-A tail of said target mRNA sequence.
84. The method of claim 72, wherein said chain terminator [comprises one of nucleotide deprivation and] is a nucleotide analog.
106. A method for detecting a target protein in a test sample, the method comprising:
 - (a) covalently attaching the target protein to an abortive promoter cassette

[(APC)] by a reactive abortive promoter cassette [APC] linker, wherein said abortive promoter cassette [APC] comprises a region that can be detected by transcription by a polymerase;

(b) incubating said target protein with an RNA-polymerase, an initiator, and a terminator;

(c) synthesizing an oligonucleotide transcript that is complementary to the transcription initiation start site of [APC] the abortive promoter cassette, wherein said initiator is extended until said terminator is incorporated into said oligonucleotide transcript, thereby synthesizing multiple reiterative oligonucleotide transcripts; and

(d) determining the presence or absence of the target protein by detecting or quantifying said [reiteratively synthesized] reiterative oligonucleotide transcripts [synthesized from said test sample].

109. The method of claim 106, wherein said [APC] abortive promoter cassette linker [will be] is covalently attached to the target protein by [modification of] thiol-reactive or amine-reactive protein crosslinking agents.

113. A method for detecting pathogens, said method comprising [the steps of]:

(a) obtaining a sample in need of detection of a pathogen

(b) hybridizing a single stranded target pathogen polynucleotide in said sample with an abortive promoter cassette comprising a nucleotide sequence that hybridizes to single stranded target pathogen polynucleotide, and a region that can be detected by transcription by a polymerase;

(c) incubating said target polynucleotide and initiator with an RNA[-]polymerase, and a terminator;

(d) synthesizing an oligonucleotide transcript that is complementary to the initiation start site of the [APC] abortive promoter cassette, wherein said initiator is extended until said terminator is incorporated into said oligonucleotides thereby synthesizing multiple abortive reiterative oligonucleotide transcripts; and

(e) determining the presence of a pathogen by detecting or quantifying said reiteratively synthesized oligonucleotide transcripts synthesized from said sample.

130. The method of claim [112 or] 113, wherein said sample is obtained from the group consisting of: animal, plant or human tissue, blood, saliva, semen, urine, sera, cerebral or spinal fluid, pleural fluid, lymph, sputum, fluid from breast lavage, mucosoal secretions, animal solids, stool, cultures of microorganisms, liquid and solid food and [feedproducts] feed-products, waste, cosmetics, air and water.

135. The method of any one of claims [1, 13, 26, 27, 41, 54-56,] 55, 56, 71, 72, [85, 101-103,] 106, [112,] or 113, [or 115,] wherein said initiator is selected from the group consisting of: nucleosides, nucleoside analogs, nucleotides, [an] and nucleotide analogs.

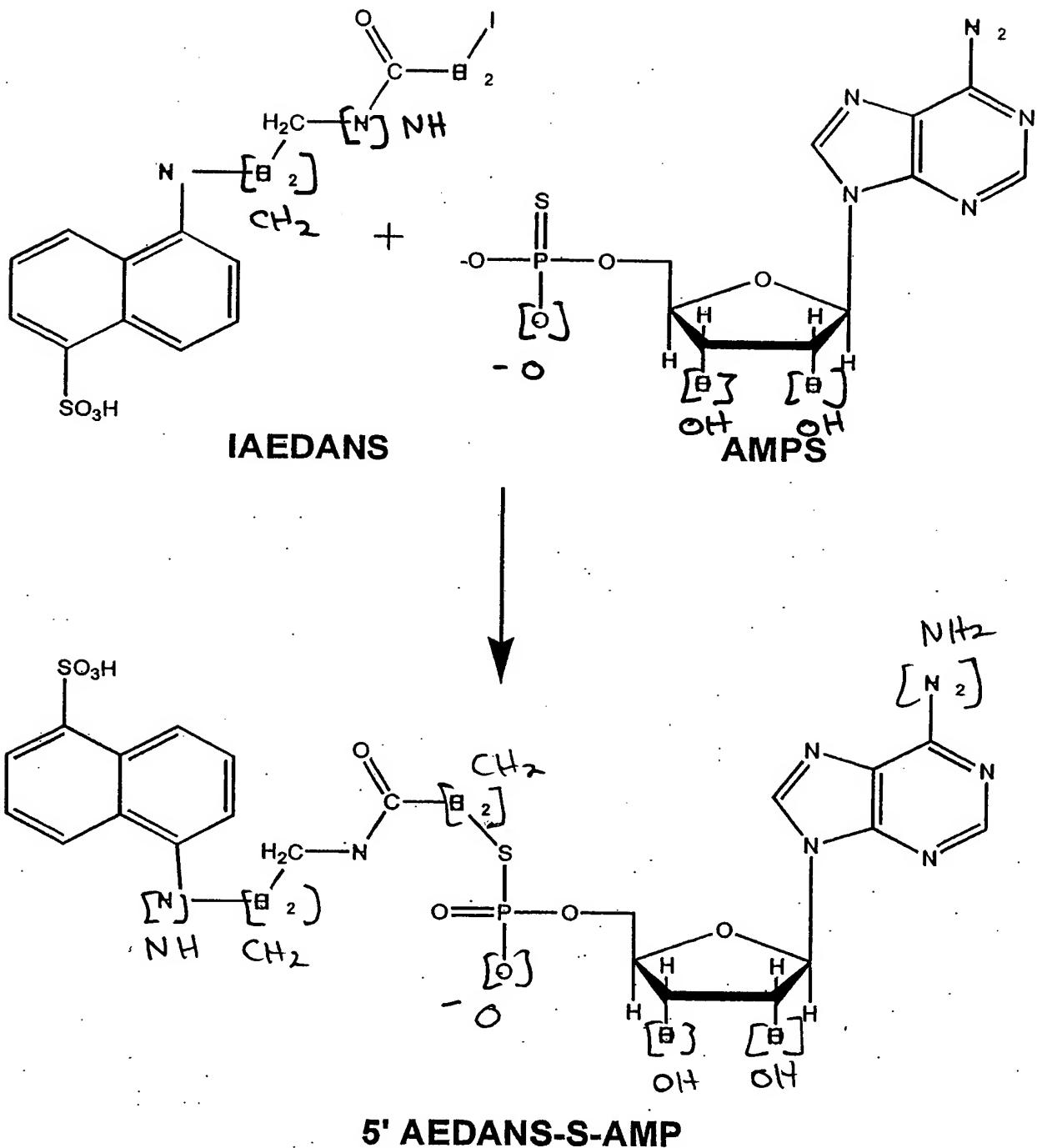


FIGURE 3

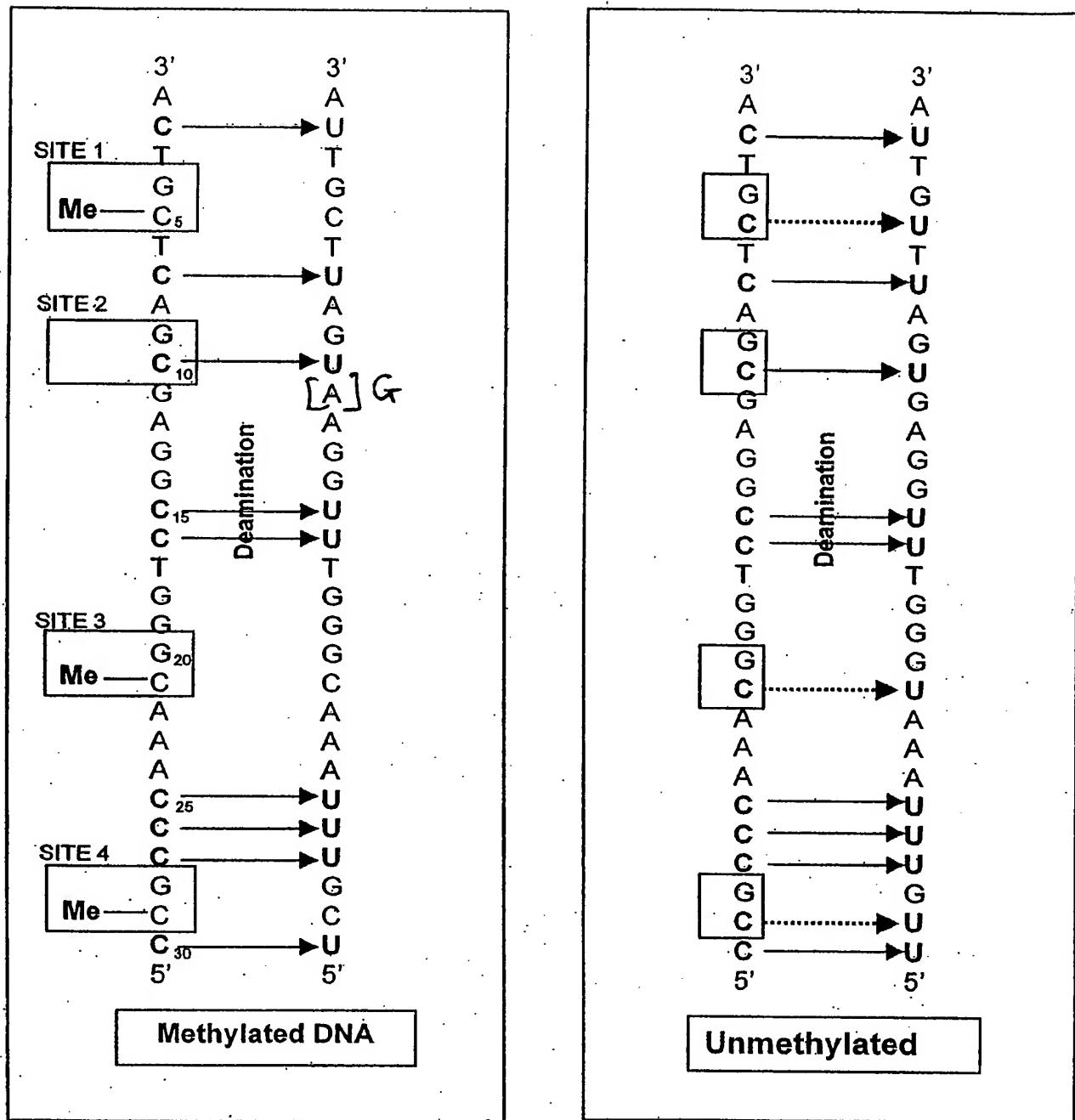
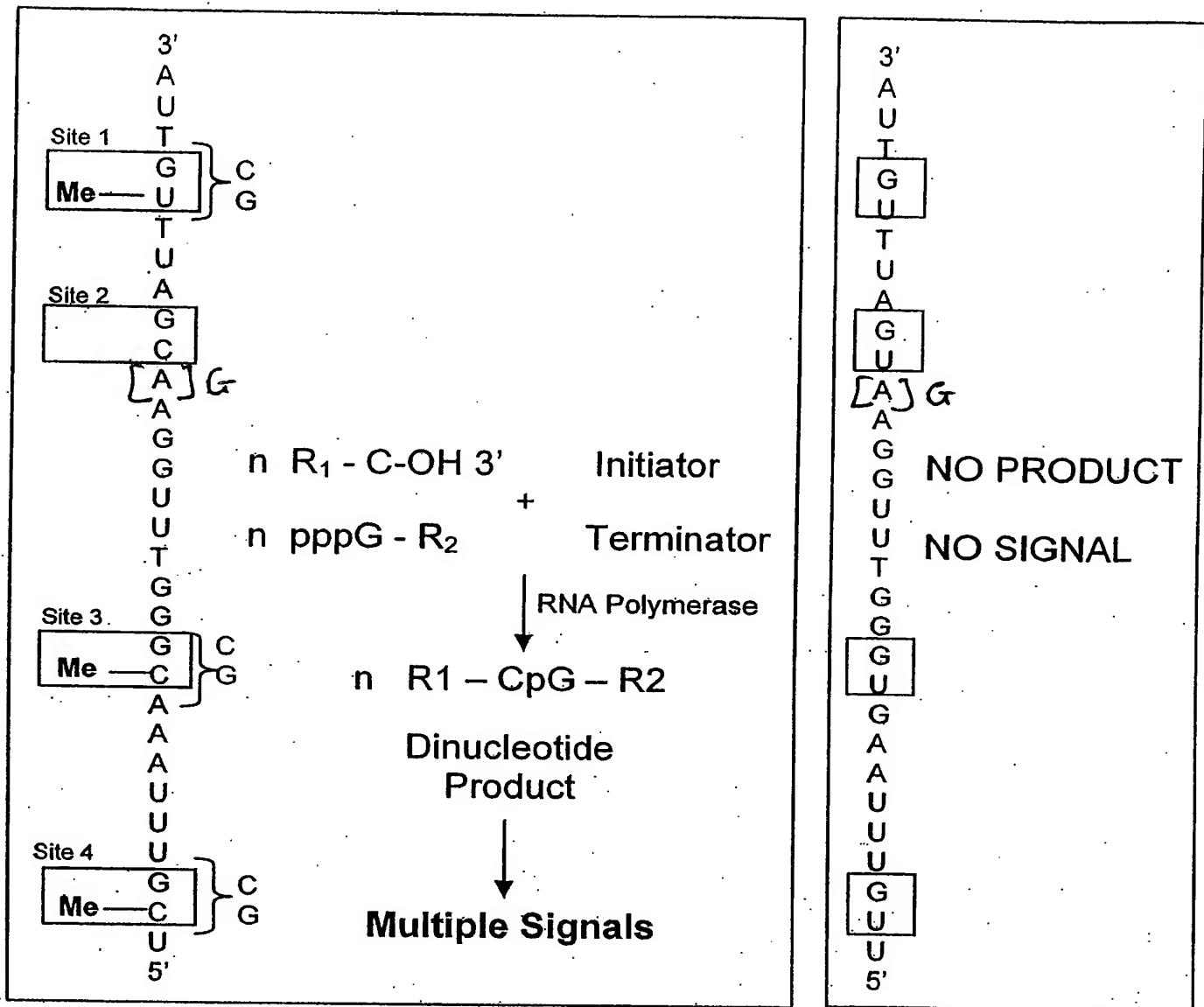


FIGURE 13

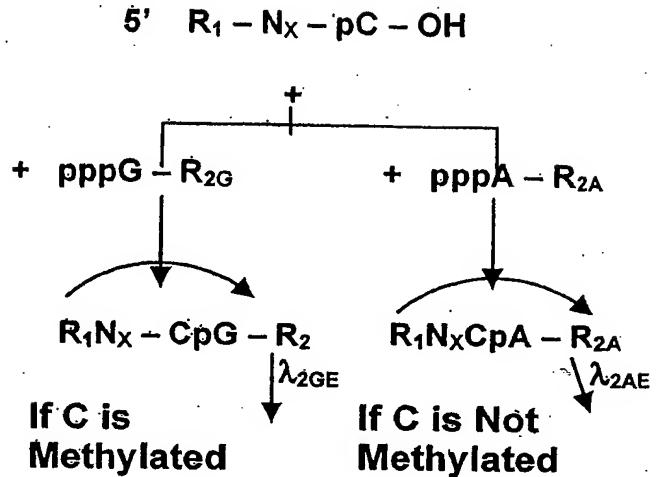
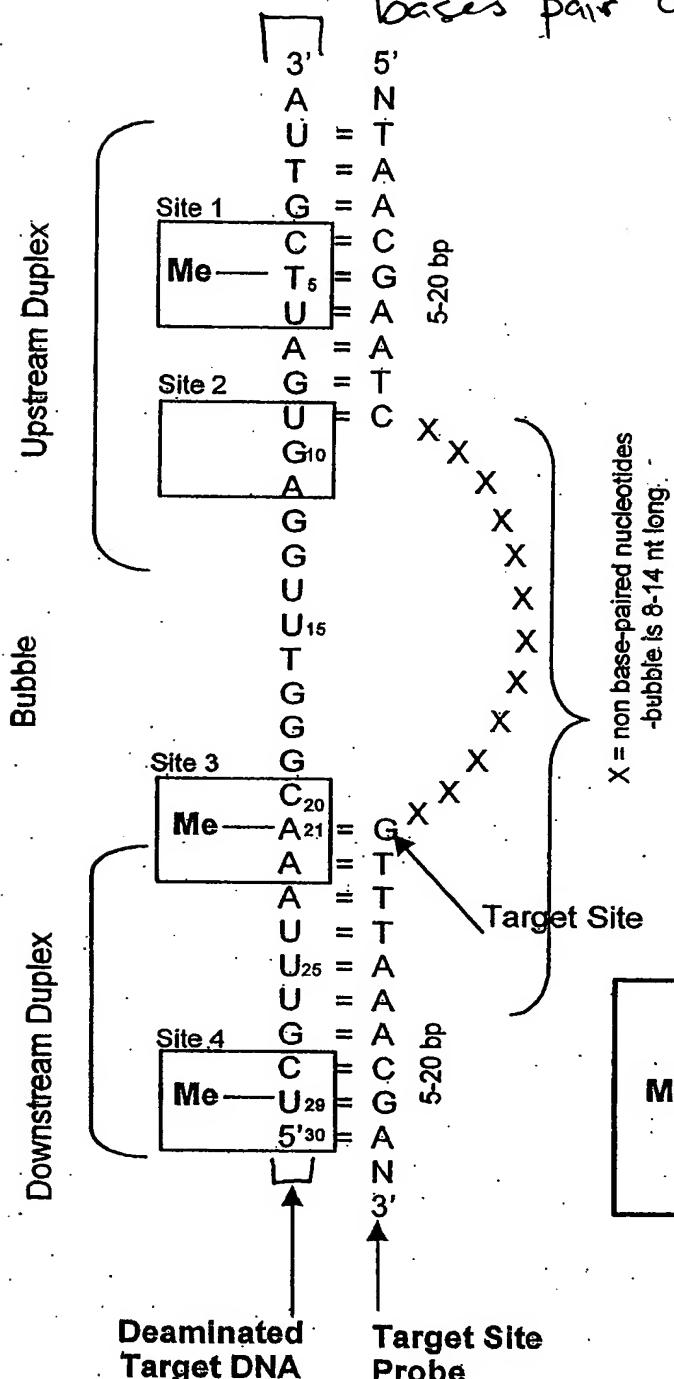


Deaminated Methylated DNA

Deaminated
Unmethylated
DNA

FIGURE 14

entire strand
shifted down by 1 nt., so that
bases pair correctly.



- M = 1 If both copies are 100% methylated: Only λ_{2GE} detected
- M = 0.5 If 1 copy is methylated: Both λ_{2GE} and λ_{2AE} detected
- M = 0 If both copies unmethylated: only λ_{2AE} detected

$$M = \text{Methylation Index} = \frac{E\lambda_{2GE}}{E\lambda_{2GE} + E\lambda_{2GA}}$$

FIGURE 15

Figure 30

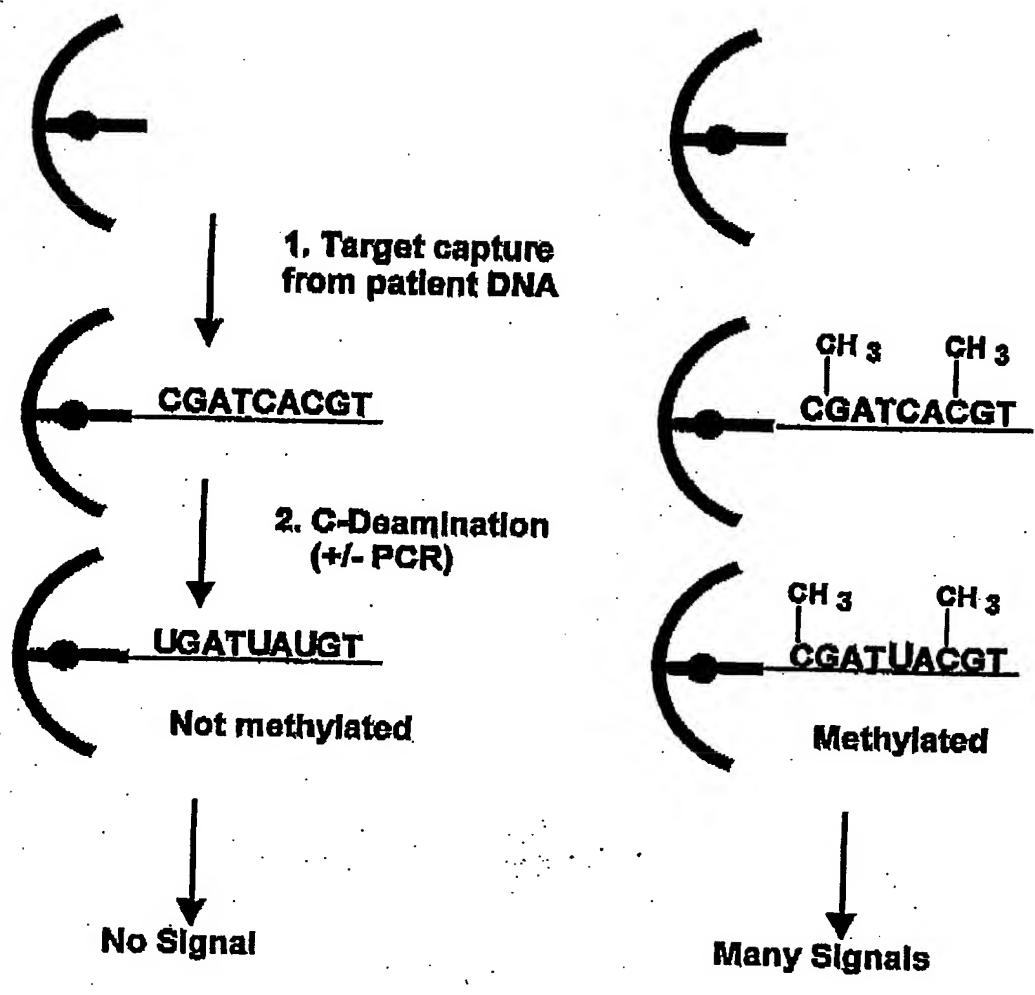


FIGURE [31]
30